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A histologic and immunohistochemical analysis of defective vaginal healing after continence taping procedures: A prospective case-controlled pilot study

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Objective: The purpose of this study was to investigate vaginal rejection of polypropylene mesh after continence taping procedures.
Study design: Of 700 women who had undergone the procedures, 17 women with sling erosion and 7 women with voiding difficulty or symptomatic vagina prolapse (control subjects) underwent histopathologic evaluation and immunohistochemistry.
Results: Seven women whose condition was not responding to conservative treatment and debridements had the exposed suburethral tape excised, which revealed predominant foreign body reaction and fragmented mesh that was surrounded by histiocytes and dense fibrosis. Immunohistochemical analysis revealed that the cell density percentage of CD^{20+} cells was statistically significantly greater in the persistent defective healing group than in either the single-debridement or control group ($P = .014$ and $P = .014$, respectively). We found statistically significant differences between the persistent defective healing and single-debridement groups and between the former and control groups in the ratios of T and B cells ($P = .035$ and $P = .022$, respectively).
Conclusion: The rate of defective vaginal healing after the procedures was 2.4%. Removal of the prosthesis and surrounding tissue at various times for the 7 women resulted in histopathologic findings that suggested a immunologic reaction. The rate of persistent defective healing of the vagina was 1%.

The tension-free vaginal tape (TVT) procedure for treatment of urinary stress incontinence in women was first reported in 1995 by Ulmsten and Petros[1] and has proved safe and effective for urinary stress incontinence, by being minimally invasive with good long-term results.[2-4]

Prolene tape seems unusually biocompatible when used as a suburethral sling. In the largest follow-up series to date (1455 TVT procedures) vaginal defects were found in only 10 cases (0.7%), with no tape rejection or infection that required tape removal.[5] Experience at our institution suggests that there is

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a higher rate than reported in the literature and that several mechanisms for defective healing of the vaginal mucosa may exist. Conservative treatment and debridements were not always able to repair the vaginal wound completely until the sling of the suburethral part was excised, then the wound healed spontaneously without additional repair.

Little is known about polypropylene mesh rejection after continence taping procedures; in particular, there is a lack of histopathologic analysis. We conducted a prospective, case-controlled pilot study to investigate the pathogenesis of vaginal rejection reaction against the prosthetic mesh.

Material and methods

The institutional review board approved the study. Between December 1995 and December 2002, 700 women aged 32 to 76 years (mean, 52.7 years) with urinary stress incontinence underwent either the TVT or the supra pubic Arc (SPARC) sling procedure. The devices were manufactured by Gynecare, a division of Ethicon (TVT; Gynecare) or American Medical System (SPARC; American Medical Systems, Inc, Minnetonka, Minn). Exclusion criteria for the present study were a fasting blood sugar level of $\geq$180 mg/dL, a postprandial sugar level of $\geq$230mg/dL, a HbA1c level of $\geq$10%, and a vaginal infection. (None of the 700 patients met the exclusion criteria for the present study.) In all cases, the TVT procedure was performed as described by Ulmsten and Petros,[1] and the SPARC procedure was performed as described by Plzak and Staskin.[6] In the operating room, before being draped, the lower abdomen and the vulvovaginal area were disinfected with 10% povidone iodine aqua solution. An additional similar disinfecting procedure of the same area was instituted immediately before the start of the continence taping procedures. For antibiotic prophylaxis, cefazolin (500 mg) was given intravenously immediately after the commencement of the procedures for all subjects.

Follow-up visits were at 1 week, 1 to 3 months, 6 months, and annually after the operation. All subjects were in compliance except for 21 women (3%) who attended only the first 3 follow-up visits, but they were also included in this study. Of the 21 women, 11 women moved overseas; 5 women had a long travel distance, and 6 women were lost of follow-up. Fifteen of the 670 TVT patients and 2 of the 30 SPARC patients had sling erosion (defective vaginal healing).

Conservative treatment, including sitz bath with warm saline solution and the local application of neomycin sulfate ointment, was first prescribed for these 17 patients once the sling erosion was noted. Patients whose condition failed to respond to the conservative treatment then underwent 1 or 2 debridements. Debridement was defined as excision of the inflammatory/granulation tissue and simple closure of the vaginal wound with the suburethral part of the tape embedded. Of the 17 patients, 4 conditions (23.5%) responded to the conservative treatment, and the women were removed from further analysis. Seven women who had undergone the TVT procedure previously (5 women with symptomatic prolapse and 2 women with voiding difficulty but without sling erosion) were derived from the 670 women as control subjects. The redundant anterior vaginal wall that covered the previously implanted prosthesis was excised and sent for examination. These 20 subjects were allocated into the persistent defective healing (PDH) group (7 patients), single-debridement (SD) group (6 patients), and control group (7 patients). Of the 20 subjects, 3 women were postmenopausal and had systemic hormone replacement therapy. The surgical pathologists were blinded to the clinical course and indications for the biopsy.

All specimens were sent for light microscopy, immunohistochemistry evaluation and Gram stain. All specimens were fixed in 10% buffered formalin, embedded in paraffin, and stained with hematoxylin-eosin. Immunohistochemical analysis was performed on 4-μm–thick sections of this tissue by the streptavidin-biotin-peroxidase method (Zymed Laboratories Inc, San Francisco, Calif). A microwave antigen retrieval was performed with monoclonal antibodies against the following antigens: CD4 (Dako, Copenhagen, Denmark; diluted 1:80), CD8 (Dako; diluted 1:160), CD20 (Dako; diluted 1:800), and CD68 (Dako; diluted 1:800).[7] Negative controls consisted of the replacement of primary antibodies by nonimmune sera.

Under a transmission light microscope (Nikon E 600; Nikon, Kanagawa, Japan), with a $\times$20 objective and $\times$10 ocular, a transparent metric ruler was placed on top of the slide (immunohistochemistry staining) for measuring the specimens. For each biopsy, the entire specimen was surveyed, and the number of lymphocytes and macrophages within the stroma was semiquantified. Cells that were located within blood vessels, within the background staining, or without nucleus that was demonstrated were not counted. The hematoxylin-eosin stained slide was checked additionally when the cell morphologic condition for the immunohistochemistry staining was uncertain.

Statistical analysis

We analyzed the data from the aforementioned analysis for those 20 patients who completed the study, using the Statistical Package for the Social Sciences (version 10.0; SPSS Inc, Chicago, Ill). A probability value of $<.05$ was considered statistically significant. The data are presented as median (range). The Kruskal-Wallis test was used to compare differences among the 3 groups and

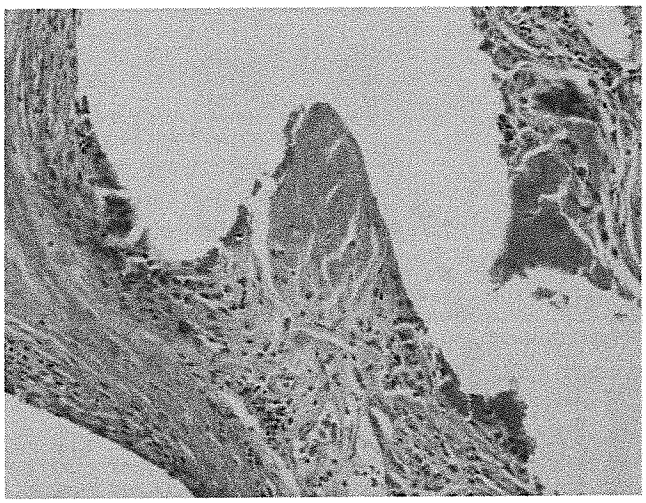

Figure 1 Palisading histiocytes, multinucleated foreign body giant cells, and lymphocytes outline the mesh constituent. (Hematoxylin-eosin stain; original magnification, ×100.)

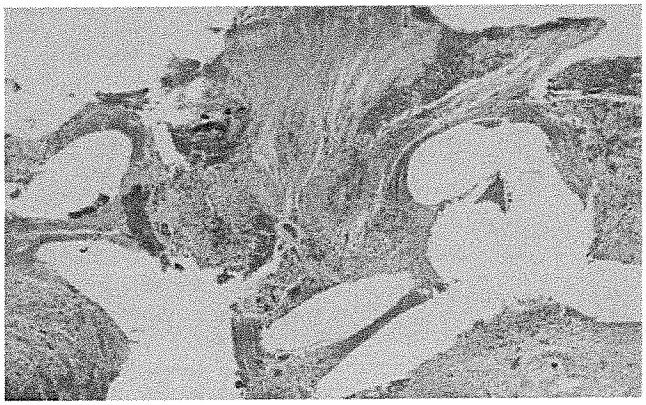

Figure 2 Polypropylene mesh 10 weeks after transvaginal implantation, with marked fibrosis around the fragmented mesh filaments. Acute and chronic inflammation at the interface of mesh filaments is also demonstrated. (Hematoxylin-eosin stain; original magnification, ×40.)

was followed by the Mann-Whitney *U* test, if the overall difference was statistically significant. However, to avoid an enlarged type I error, in the significance of multiple comparisons, a probability value of < .017 was considered statistically significant. For intragroup comparison, the Wilcoxon signed-rank test was used.

Results

We found a 2.4% (17 of 700 patients) rate of defective vaginal healing. The period from implantation to discovery of the defective healing varied from 1 to 3 months (mean, 2.4 months). Of those subjects who were aged 37 to 62 years (mean, 41.2 years), (5) women complained of pain, wound tissue granulation, and increased vaginal discharge; (4) women complained of dyspareunia by themselves or partner discomfort during

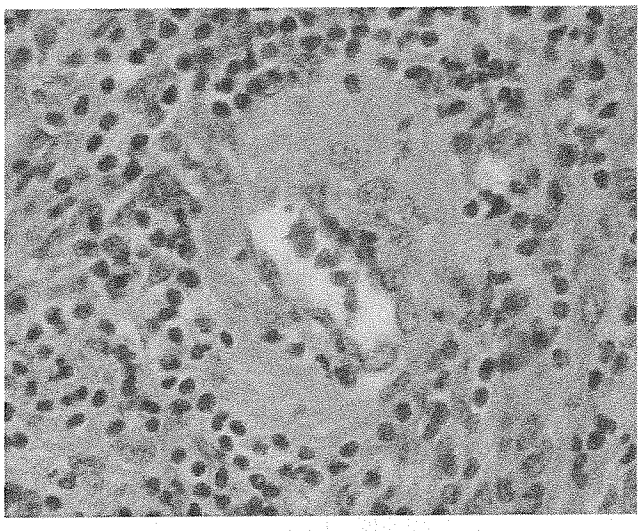

Figure 3 Perivascular inflammatory cell infiltrate that includes lymphocytes and eosinophils occupies the fibrotic stroma. (Hematoxylin-eosin stain; original magnification, ×400.)

sexual intercourse, and (5) women complained of vaginal bleeding and irritative voiding. Another 3 subjects were asymptomatic, and sling erosion was discovered at routine follow-up 2 months after the operation. The defectively healed wounds were located exclusively suburethrally; no case was noted elsewhere, including the suprapubic area or other tissue covering the sling.

Complete epithelialization over the mesh occurred 1 month after conservative treatment in 4 of these 17 subjects (3 subjects in the TVT group and 1 subject in the SPARC group). Because the vaginal wall failed to heal 4 weeks after 2 debridements, 7 of the 17 subjects required excision of the exposed suburethral part of the sling (ca, 1 × 0.5 cm), whereas the remaining 6 patients needed only 1 debridement to repair the vaginal wound. The vaginal wounds of these 7 subjects healed spontaneously without additional repair after sling explantation, which resulted in a 1% rate of PDH. Four of 7 patients (57%) who had the sling tape removed remained dry (stress continent) after a 12- to 84-month (mean, 68.2 months) follow-up. The difference of the rates of defective healing in TVT and SPARC groups was not statistically significant (2.2% vs 6.7%; $P = .162$; Fisher's exact test).

Persistent defective healing group (patients 1 to 7)

The significant histologic features in this group showed that the mesh filaments were fragmented and surrounded by palisading histiocytes and occasional multinucleated giant cells. The inflammatory process was accompanied by pronounced perifilamentous fibrosis and a predominant foreign body reaction (Figures 1

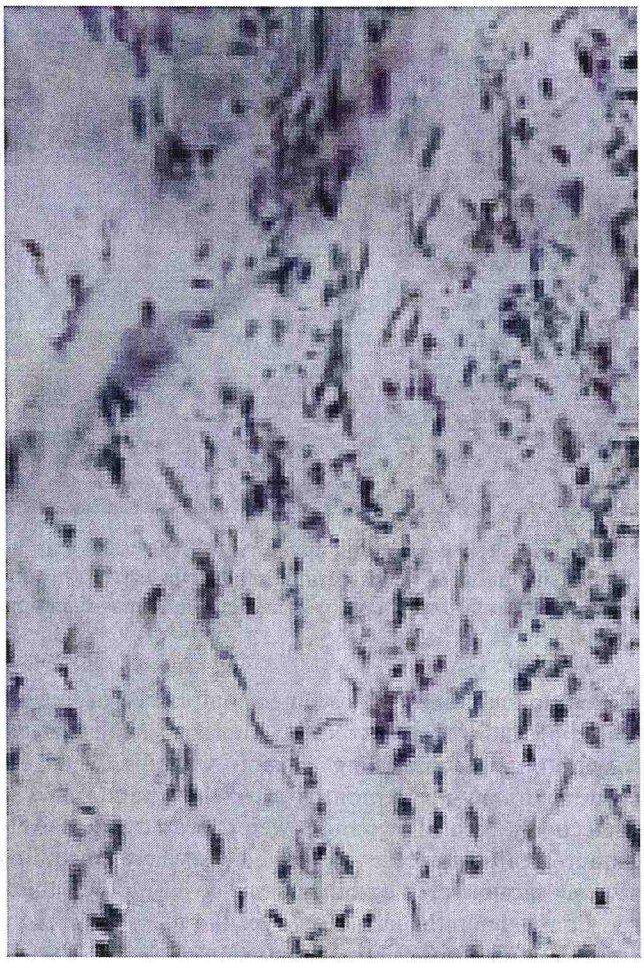

Figure 4 CD^{20+} B-lymphocytes formed small aggregates around the tape filament. (Original magnification, ×40.)

and 2) and by occasional perivascular mononuclear cell infiltration (Figure 3). The presence of this feature means that, by the time the delayed-type hypersensitivity response is developed after being previously sensitized through an unknown exposure, cytokines elaborated by T_{H1} response cells induce blood monocytes to adhere to vascular endothelial cells and migrate from the blood into the surrounding tissues. During this process the monocytes differentiate into activated macrophages.[8] Immunohistochemistry staining showed that B cells (CD^{20+}) formed small aggregates around the mesh filaments or were distributed randomly in the stroma (Figure 4). CD^{4+} T cells were distributed in the fibrotic stroma around mesh filaments. CD^{8+} T cells were also present in the fibrotic stroma. The mesh filaments were surrounded by CD^{68+} histiocytes and multinucleated foreign body giant cells.

Single-debridement group (patients 8 to 13)

The hematoxylin-eosin staining for these 6 subjects showed granulation tissue with variable degrees of acute and chronic inflammation and fibrosis; only 1 subject showed foreign body reaction. Immunohistochemistry staining showed the presence of CD^{4+}, CD^{8+}, and CD^{20+} cells; CD68+ cells also were present randomly in the submucosa. The number of these cells varied; there were no specific distribution patterns.

Table I Patient characteristics and histopathologic features

Patient	Age (y)	Menopause	Histologic feature
1	42	No	Multinucleated giant cells fibrosis, chronic inflammation, foreign body reaction
2	50	No	Fibrosis, congestion, chronic inflammation, foreign body reaction
3	54	No	Acute and chronic inflammation, foreign body reaction, fibrosis
4	62	Yes	Acute and chronic inflammation, hemorrhage, fibrosis, foreign body reaction
5	43	No	Granulation tissue, acute and chronic inflammation, foreign body reaction, fibrosis
6	42	No	Granulation tissue, acute and chronic inflammation, foreign body reaction, edema, hemorrhage
7	71	No	Granulation tissue, acute and chronic inflammation, foreign body reaction, fibrosis; purulent exudate on the tape
8	37	No	Dense fibrosis, acute and chronic inflammation, congestion
9	42	No	Fibrosis, edema, acute and chronic inflammation
10	46	No	Granulation tissue, fibrosis, acute and chronic inflammation, hemorrhage
11	42	No	Granulation tissue, acute and chronic inflammation, hemorrhage
12	40	No	Acute and chronic inflammation, fibrosis, foreign body reaction
13	53	No	Acute and chronic inflammation, fibrosis
14	58	Yes	Unremarkable vaginal wall, mild lymphocytic infiltration
15	47	No	Congestion, chronic inflammation
16	56	Yes	Chronic inflammation
17	41	No	Unremarkable vaginal wall
18	38	No	Chronic inflammation, congestion
19	35	No	Unremarkable vaginal wall
20	40	No	Chronic inflammation, congestion

No micro-organisms were found in any Gram stains of the 20 patients, except Gram-positive staphylococcus was noted on the tape fiber of patient 7. Patients 1-7, persistent defective healing group; patients 8-13, single-debridement group; patients 14-20, control group.

Table II Comparison of cell density percentage for different antibodies in immunohistochemical study

	Persistent defective healing group (n = 7)	Single-debridement group (n = 6)	Control group (n = 7)	*P* value			
				Over all	Persistent defective healing group vs single-debridement group	Persistent defective healing group vs control group	Single-debridement group vs control group
CD^4	17.5 (3.1-28.4)	17.6 (7.5-33.4)	14.7 (10.0-41.1)	.843			
CD^8	19.0 (9.7-46.4)	13.3 (8.4-35.5)	20.0 (12.7-31.0)	.612			
CD^{20}	26.1 (19.2-34.6)	10.5 (6.2-30.3)	11.8 (6.1-29.3)	.020	.014	.014	1.00
CD^{68}	36.7 (10.9-47.8)	44.4 (19.1-77.2)	43.7 (27.8-66.1)	.338			
$CD^4 + CD^8/CD^{20}$	1.7 (0.5-2.7)	2.5 (1.7-6.2)	3.2 (1.3-6.7)	.034	.035	.022	.62
$CD^4 + CD^8/CD^4 + CD^8 + CD^{20}$	0.6 (0.3-0.7)	0.7 (0.6-0.9)	0.8 (0.6-0.9)	.034	.035	.022	.62

Data (unit: total number of cells/cm^2 %) are presented as the median (range). The probability value denotes overall comparison among 3 groups by the Kruskal-Wallis test or pairwise comparison with the Mann-Whitney *U* test.

Control group (patients 14 to 20)

Hematoxylin-eosin stainings for the 7 control patients, whose range of age, menopause status, and body mass index were similar to study group, showed mild mononuclear cell infiltration without submucosa fibrosis. Immunohistochemistry stainings showed that most of the inflammatory cells were CD^{4+} cells; some CD^{8+} and CD^{20+} cells were also noted. CD^{68+} macrophages were distributed randomly in the submucosa. Table I summarizes the histologic findings for each patient.

Immunohistochemistry evaluation

In the PDH group, there was a persistently sustained number of immunocytes and a predominant infiltration of CD^{20+} lymphocytes. Compared with either CD^{4+} or CD^{8+} cells, patients in this group, although not significantly different, had higher humoral immunity in CD^{20+} cells ($P = .18$, $P = .09$, respectively; Wilcoxon signed-rank test). When immunomodulation for the correlation between T and B cells was investigated, we found statistically significant differences between the PDH and SD groups and between the PDH and control groups in the ratios of T and B cells and T and T plus B cells ($P = .035$, $P = .022$, respectively; $P = .035$, $P = .022$, respectively; Table II).

For a short-term inflammatory reaction (within 4 weeks after implantation), the SD group showed relatively higher levels of CD^{68+} cell-mediated inflammation and a relatively greater number of CD^{4+} T cells. Because there was no significant difference when compared with the control group in T- and B-cell connection ($P = .62$), those tissue might be in acute inflammatory status. For those patients with persistent inflammatory reaction (over 8 weeks after implantation), a comparison of the PDH with both the control and SD groups revealed that the number of CD^{20+} cells was statistically significant greater in the PDH group ($P = .014$, $P = .014$, respectively; Table II).

Comment

We report a 2.4% rate of defective vaginal healing and a 1% incidence of PDH of the anterior vagina, 1 to 7 years after the operation. In contrast with other synthetic meshes that are used in the TVT procedure, rejection of polypropylene mesh, a type 1 nonabsorbable monofilament macroporous prosthesis that was used in the TVT and SPARC procedures, has never been reported,[1,2,4,5] with the exception of only 3 cases that were unconfirmed by morphologic study.[9]

Vaginal erosion may occur after delayed infection of the synthetic sling or prominent foreign body reaction, which leads to separation of the vaginal incision and sling erosion.[10,11] Six women had complete epithelialization over the mesh after 1 debridement of the vaginal tissue. We attribute these results to the presence of factors such as inadequate vaginal tissue coverage during the operation,[12] rigidity of the mesh and its propensity for injury to adjacent tissues, or a site-specific localized inflammatory response of the suburethral vagina.[13,14] Follow-up study is still ongoing to investigate whether a delayed hypersensitivity host versus prosthesis antigen-antibody response exists.

Together with the evidence of spontaneous epithelialization of the vaginal wound after sling explantation, the most striking finding for the other 7 women who underwent double debridements was that the sling constituents were fragmented and had predominant foreign body reaction, dense fibrosis, and occasional

perivascular mononuclear cell infiltration. In comparison with the SD and control groups, the PDH group revealed significant differences in immunohistochemistry analyses, namely the regression of cell-surface markers (CD^{20+}) in the progression of the immune response. The regional analysis of cell-surface marker expression patterns provides a window on immunoreactivity and pathogenesis of prosthesis rejection. Inflammatory cells and mediators that generate after tissue injury, infection, or inflammation serve as initiators of events that may culminate in the generation of productive T- and B-cell responses and long-term immunity.[15] Mature CD^4 T cells in the graft that are activated by the pathogen produce a "cytokine storm" that recruits other T cells, macrophages, and natural killer cells to create the inflammation characteristic of graft versus host response.[16]

Of 24 women who underwent sling removal in the report of Bent et al,[17] the reaction site was in the vagina (18 women), the abdomen (8 women), and both sites (2 patients); however, in the present study, the reaction site was exclusively in the suburethral vaginal wall. After vaginal implantation of polypropylene mesh for urinary stress incontinence, Bryans[18] reported a 7.2% vaginal nonhealing rate, and Drutz et al[19] reported a 6.2% rate. However, the incidence of sling erosion reported by Ward et al[20] was 1 of 170 (0.6%) and by Kuuva and Nilsson[5] was 10 of 1455 (0.7%) for women who had undergone the TVT procedure. A plausible reason for this very low rate is the underestimation of morbidity, possibly because of asymptomatic erosion or limited follow-up or nondiscovery of the lesion. Thus, physician awareness during postoperative vaginal examination is mandatory.

In our series, none of 17 women had bladder or urethral involvement, but 13 women needed either debridement(s) or sling explantation. In contrast, Kobashi and Govier[21] noted that 4 women with mesh erosion and minimal granulation tissue reaction after the continence taping procedure (polypropylene) needed only sexual abstinence and conservative observation. However, both studies concluded imminent sling explantation that once was diagnosed as not necessary. The outcome of these 2 studies raises questions regarding what is a reasonable time frame and the conditions of local disease to consider for conservative or surgical treatment.

The results of this pilot study suggest that polypropylene mesh is not always biologically inert. Because a large and still increasing number of continence taping procedures have been undertaken, there is a clinical and theoretic basis for concern. Prospective epidemiologic and further studies that correlate the biocompatibility of polypropylene mesh and its degradation products with clinical findings are anticipated.

In this pilot study, we have demonstrated in 7 patients that, to our knowledge, there is possibly a previously unreported immunologic reaction of the vagina to polypropylene mesh. Removal of the prosthesis and surrounding tissue at various times resulted in microscopic and immunohistochemistry findings that suggested a host versus prosthesis reaction. In contrast, the other groups displayed a range of acute and chronic inflammation or unremarkable findings. The rate of PDH after the continence taping procedures was 1% (7/700 women).

Acknowledgments

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